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☐ 1. Document ID: US 20040005678 A1

Using default format because multiple data bases are involved.

L1: Entry 1 of 5 File: PGPB Jan 8, 2004

PGPUB-DOCUMENT-NUMBER: 20040005678
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040005678 A1

TITLE: Biosynthesis of amorpha-4,11-diene

PUBLICATION-DATE: January 8, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Keasling, Jay	Berkeley	CA	US	
Martin, Vincent	Kensington	CA	US	
Pitera, Douglas	Oakland	CA	US	
Withers, Sydnor T. III	Richmond	CA	US	
Newman, Jack	Berkeley	CA	US	

US-CL-CURRENT: 435/146; 435/193, 435/252.3, 435/320.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 2. Document ID: US 20030148479 A1

L1: Entry 2 of 5 File: PGPB Aug 7, 2003

PGPUB-DOCUMENT-NUMBER: 20030148479
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030148479 A1

TITLE: Biosynthesis of isopentenyl pyrophosphate

PUBLICATION-DATE: August 7, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Keasling, Jay	Berkeley	CA	US	
Martin, Vincent	Kensington	CA	US	

Pitera, Douglas	Berkeley	CA	US
Kim, Seon-Won	Jeongdong-myeon Sacheon	CA	KR
Withers, Sydnor T. III	Richmond	CA	US
Yoshikuni, Yasuo	Berkeley	CA	US
Newman, Jack	San Francisco	CA	US
Khlebnikov, Artem Valentinovich	Mountain View		US

US-CL-CURRENT: 435/131; 435/252.3, 435/320.1, 435/471

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 3. Document ID: EP 982404 A1

L1: Entry 3 of 5 File: EPAB Mar 1, 2000

PUB-NO: EP000982404A1
DOCUMENT-IDENTIFIER: EP 982404 A1
TITLE: DNA encoding amorpha-4,11-diene synthase

PUBN-DATE: March 1, 2000

INVENTOR-INFORMATION:

NAME	COUNTRY
WALLAART, THORVALD EELCO DRS	NL
BOUWMEESTER, HENDRIK JAN DR IR	NL

INT-CL (IPC): C12 N 15/60; C12 N 15/70; C12 N 15/82; C12 N 9/88; C12 N 5/10; C12 N 1/21; C12 P 5/00; C12 P 17/18; A01 H 5/00
EUR-CL (EPC): C12N009/88; C12N015/82, C12N015/82 , C12P005/00 , C12P017/18 , C12P017/18

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 4. Document ID: US 20040005678 A1

L1: Entry 4 of 5 File: DWPI Jan 8, 2004

DERWENT-ACC-NO: 2004-120864
DERWENT-WEEK: 200432
COPYRIGHT 2004 DERWENT INFORMATION LTD
TITLE: Synthesizing amorpha-4,11-diene in a host cell, useful as pharmaceuticals, comprises introducing nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate

INVENTOR: KEASLING, J; MARTIN, V ; NEWMAN, J ; PITERA, D ; WITHERS, S T

PRIORITY-DATA: 2003US-0411066 (April 9, 2003), 2001US-0006909 (December 6, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC

US 20040005678 A1 January 8, 2004 075 C12P007/42

INT-CL (IPC): C07 H 21/04; C12 N 1/21; C12 N 9/10; C12 N 15/74; C12 P 7/42

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 5. Document ID: AU 766764 B, EP 982404 A1, WO 200012725 A2, AU 9957423 A, EP 1108041 A2, BR 9913196 A, ZA 200101455 A, CN 1321194 A, JP 2002523101 W, MX 2001002040 A1

L1: Entry 5 of 5 File: DWPI Oct 23, 2003

DERWENT-ACC-NO: 2000-258617
DERWENT-WEEK: 200381

TITLE: New isolated DNA sequences and polypeptides comprising amorpha-4,11-diene synthase activity, useful for production of amorphadiene and/or artemisinin

INVENTOR: BOUWMEESTER, H J; WALLAART, T E ; WALLAART, T E D

PRIORITY-DATA: 1998EP-0202854 (August 27, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 766764 B	October 23, 2003		000	C12N015/60
EP 982404 A1	March 1, 2000	E	041	C12N015/60
WO 200012725 A2	March 9, 2000	E	000	C12N015/60
AU 9957423 A	March 21, 2000		000	C12N015/60
EP 1108041 A2	June 20, 2001	E	000	C12N015/60
BR 9913196 A	September 25, 2001		000	C12N015/60
ZA 200101455 A	October 31, 2001		060	C12N000/00
CN 1321194 A	November 7, 2001		000	C12N015/60
JP 2002523101 W	July 30, 2002		053	C12N015/09
MX 2001002040 A1	May 1, 2002		000	A01H005/00

INT-CL (IPC): A01 H 5/00; C12 N 0/00; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/04; C12 N 9/88; C12 N 15/09; C12 N 15/60; C12 N 15/70; C12 N 15/82; C12 P 5/00; C12 P 17/18; C12 R 1/19; C12 R 1/84; C12 R 1/865; C12 R 1:19; C12 R 1:84; C12 R 1:865; C12 R 1/19; C12 R 1:84; C12 R 1:865; C12 N 1/19; C12 N 1/19; C12 N 9/04; C12 N 9/04; C12 N 9/04; C12 N 9/04; C12 N 9/04; C12 N 9/88; C12 N 9/88; C12 N 9/88; C12 N 9/88 ; C12 N 9/88; C12 N 15/09; C12 P 5/00; C12 P 5/00; C12 P 5/00; C12 P 5/00; C12 P 5/00; C12 P 17/18; C12 P 17/18; C12 P 17/18; C12 P 17/18; C12 P 17/18; C12 R 1:19; C12 R 1:19; C12 R 1:19; C12 R 1:645; C12 R 1:645; C12 R 1:645; C12 R 1:645; C12 R 1:645; C12 R 1:84; C12 R 1:84; C12 R 1:84; C12 R 1:84; C12 R 1:865; C12 R 1:865; C12 R 1:865; C12 R 1:865; C12 R 1:91; C12 R 1:91; C12 R 1:91; C12 R 1:91; C12 R 1:91

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

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Terms	Documents
amorpha-4,11-diene synthase	5

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WEST Search History

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DATE: Tuesday, June 08, 2004

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☐	L13	amorphadiene	2
☐	L12	amorpha-4	0
☐	L11	amorpha-4, 11-diene and synthase	0
☐	L10	amorpha-4, 11-diene	0
☐	L9	amorpha-4 11-diene	0
☐	L8	amorpho? synthase.clm	0
☐	L7	amorpha? synthase.clm	0
☐	L6	amorphadiene synthase.clm	0
☐	L5	amorpha-4,11-diene.clm.	1
☐	L4	amorphadiene.clm.	0
	DB=PGPB; PLUR=YES; OP=ADJ		
☐	L3	US-20040005678-A1.did.	1
☐	L2	US-20040005678-A1.did.	1
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L1	amorpha-4,11-diene synthase	5

END OF SEARCH HISTORY

=> d l5 1-15 ibib ab

L5 ANSWER 1 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:18837 HCAPLUS
DOCUMENT NUMBER: 140:92683
TITLE: Preparation of **amorpha-4, 11-diene** with transgenic microorganisms producing isopentenyl- and dimethylallyl pyrophosphates
INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Withers, Sydnor T.; Newman, Jack
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 75 pp., Cont.-in-part of U.S. Ser. No. 6,909.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004005678	A1	20040108	US 2003-411066	20030409
US 2003148479	A1	20030807	US 2001-6909	20011206

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206
AB Methods for synthesizing **amorpha-4,11-diene** from isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. **Amorpha-4,11-diene** is then produced with the transgenic microorganism which is further transformed with an optimized **amorpha-4,11-diene** synthase gene. The **amorpha-4,11-diene** may be used in synthesis of the antimalarial drug artemisinin. Thus, **amorpha-4,11-diene** was prepd. from mevalonate supplied in the medium with Escherichia coli transformed with plasmid pBBRMDIS-2, contg. the yeast genes idi (for isopentenyl pyrophosphate isomerase) and ispA (for farnesyl pyrophosphate synthase) and the genes for mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, and **amorpha-4,11-diene** synthase. The yield was 2 .mu.g **amorpha-4,11-diene** /mL.

L5 ANSWER 2 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:242499 HCAPLUS
DOCUMENT NUMBER: 138:270406
TITLE: Plant enzymes for bioconversion of sesquiterpenes
INVENTOR(S): Bouwmeester, Hendrik Jan; De Kraker, Jan-Willem; Schurink, Marloes; Bino, Raoul John; De Groot, Aede; Franssen, Maurice Charles Rene
PATENT ASSIGNEE(S): Plant Research International B.V., Neth.
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003025193	A1	20030327	WO 2002-NL591	20020917

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES,

FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: EP 2001-203519 A 20010917

AB The invention provides the use of enzymes derived from plants in biocatalysis. The regio- and stereoselective introduction of an oxygen group into an unactivated org. compd. is still a largely unresolved challenge to org. chem. (Faber, 2000). We have shown that enzymes of Asteraceae species are capable of converting with high regio- and stereospecificity for example sesquiterpene olefins to com. interesting products.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:609986 HCAPLUS
DOCUMENT NUMBER: 139:160786
TITLE: Biosynthesis of isopentenyl pyrophosphate using recombinant microbial metabolic pathways
INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim, Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo; Newman, Jack; Khlebnikov, Artem Valentinovich
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 40 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003148479	A1	20030807	US 2001-6909	20011206
US 2004005678	A1	20040108	US 2003-411066	20030409

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206

AB Methods for synthesizing isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. A related method comprises introducing into a host microorganism an intermediate in the mevalonate pathway and at least one heterologous nucleic acid sequence, each sequence coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into isopentenyl pyrophosphate. The invention also provides nucleic acid sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

L5 ANSWER 4 OF 15 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2003324605 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12778056
TITLE: Engineering a mevalonate pathway in Escherichia coli for production of terpenoids.
AUTHOR: Martin Vincent J J; Pitera Douglas J; Withers Sydnor T; Newman Jack D; Keasling Jay D
CORPORATE SOURCE: Department of Chemical Engineering, 201 Gilman Hall, University of California, Berkeley, California 94720-1462, USA.
SOURCE: Nature biotechnology, (2003 Jul) 21 (7) 796-802.
Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States

DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200404
ENTRY DATE: Entered STN: 20030713
Last Updated on STN: 20040407
Entered Medline: 20040406

AB Isoprenoids are the most numerous and structurally diverse family of natural products. Terpenoids, a class of isoprenoids often isolated from plants, are used as commercial flavor and fragrance compounds and antimalarial or anticancer drugs. Because plant tissue extractions typically yield low terpenoid concentrations, we sought an alternative method to produce high-value terpenoid compounds, such as the antimalarial drug artemisinin, in a microbial host. We engineered the expression of a synthetic **amorpha-4,11-diene** synthase gene and the mevalonate isoprenoid pathway from Saccharomyces cerevisiae in Escherichia coli. Concentrations of amorphadiene, the sesquiterpene olefin precursor to artemisinin, reached 24 microg caryophyllene equivalent/ml. Because isopentenyl and dimethylallyl pyrophosphates are the universal precursors to all isoprenoids, the strains developed in this study can serve as platform hosts for the production of any terpenoid compound for which a terpene synthase gene is available.

L5 ANSWER 5 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:972642 HCAPLUS
DOCUMENT NUMBER: 139:97975
TITLE: Hydroxylation of sesquiterpenes by enzymes from chicory (Cichorium intybus L.) roots
AUTHOR(S): de Kraker, Jan-Willem; Schurink, Marloes; Franssen, Maurice C. R.; Konig, Wilfried A.; de Groot, Aede; Bouwmeester, Harro J.
CORPORATE SOURCE: Laboratory of Organic Chemistry, Wageningen University, Wageningen, 6703 HB, Neth.
SOURCE: Tetrahedron (2003), 59(3), 409-418
CODEN: TETRAB; ISSN: 0040-4020
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A microsomal enzyme prepn. of chicory roots catalyzes the hydroxylation of various sesquiterpene olefins in the presence of NADPH. Most of these hydroxylations take place at an isopropenyl or isopropylidene group. The no. of products obtained from any of the substrates is confined to one or, in a few cases, two sesquiterpene alcs. In addn., the conversion of (+)-valencene into nootkatone through .beta.-nootkatol was obsd. The involvement of (+)-germacrene A hydroxylase (a cytochrome P 450 enzyme) and other enzymes of sesquiterpene lactone biosynthesis in these reactions is discussed.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:904551 HCAPLUS
TITLE: Western Australian sandalwood oil-new constituents of Santalum spicatum (R. Br.) A. DC. (Santalaceae)
AUTHOR(S): Valder, Claudia; Neugebauer, Michael; Meier, Manfred; Kohlenberg, Birgit; Hammerschmidt, Franz-Josef; Braun, Norbert A.
CORPORATE SOURCE: Pharmazeutisches Institut, Universitaet Bonn, Bonn, D-53115, Germany
SOURCE: Journal of Essential Oil Research (2003), 15(3), 178-186
CODEN: JEOREG; ISSN: 1041-2905
PUBLISHER: Allured Publishing Corp.

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Com. Australian sandalwood oil produced from Santalum spicatum (R. Br.) A. DC. roots was analyzed using GC and GC/MS. Seventy constituents were identified: four monoterpenes, 64 sesquiterpenes and two others. Four compds. (Z)-.beta.-curcumen-12-ol, (Z)-12-hydroxysesquicineole, 6,10-epoxybisabol-2-en-12-ol and nor-helifolen-12-al were found to our knowledge for the first time in nature and were characterized using 1H-, 13C-NMR, GC/FTIR and GC/MS analyses.
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:187822 HCAPLUS
TITLE: Cloning, E. coli expression and molecular analysis of a novel sesquiterpene synthase gene from Artemisia annua
AUTHOR(S): Liu, Yan; Ye, Hechun; Li, Guofeng
CORPORATE SOURCE: Key laboratory of Plant Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, Peop. Rep. China
SOURCE: Zhiwu Xuebao (2002), 44(12), 1450-1455
CODEN: CHWHAY; ISSN: 0577-7496
PUBLISHER: Kexue Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 1 886 bp full-length sesquiterpene synthase (AaSES) cDNA was cloned from a high-yield Artemisia annua L. strain 001 by a rapid amplification of cDNA end (RACE) strategy. AaSES was 59% identical to Artemisia cyclase cDNA clone cASCl25, 50% identical to epi-cedrol synthase from A. annua, 48% identical to **amorpha-4,11-diene** synthase from A. annua, 39% identical to the 5-epi-aristolechene synthase from tobacco, 38% identical to vetispiradiene synthase from H. muticus, 41 % identical to the .delta.-cadinene synthase from cotton. The coding region of the cDNA was inserted into a procaryotic expression vector pET-30a and overexpressed in E. coli BL21 (DE3). The cyclase proteins extd. from bacterial culture were found largely in an insol. protein fraction. AaSES expressed in leaves, stems and flowers, not in roots as indicated by Northern blotting anal.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 15 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002411374 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12165305
TITLE: Volatile components from European liverworts Marsupella emarginata, M. aquatica and M. alpina.
AUTHOR: Adio Adewale Martins; Paul Claudia; Konig Wilfried A; Muhle Hermann
CORPORATE SOURCE: Institut fur Organische Chemie, Universitat Hamburg, Martin-Luther-King Platz-6, D-20146 Hamburg, Germany.
SOURCE: Phytochemistry, (2002 Sep) 61 (1) 79-91.
Journal code: 0151434. ISSN: 0031-9422.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020808
Last Updated on STN: 20021217
Entered Medline: 20021211
AB The hydrodistillation products of the liverworts Marsupella emarginata, M. aquatica and M. alpina were investigated by spectroscopic methods. A number of new compounds could be isolated by preparative gas

chromatography (GC) and identified by spectroscopic techniques including GC-mass spectrometry, NMR and chemical correlations in conjunction with enantioselective GC. From M. emarginata, in addition to many known compounds, the sesquiterpene hydrocarbon (-)-7-epi-eremophila-1(10),8,11-triene (1) and the sesquiterpene derivatives (-)-4-epi-marsupellol (2), (-)-marsupellol acetate (18), (-)-4-epi-marsupellol acetate (4), (+)-5-hydroxymarsupellol acetate (5) and (-)-9-acetoxygymnomitr-8(12)-ene (24) could be identified. In M. aquatica the sesquiterpene hydrocarbons (-)-myltayl-8(12)-ene (7), ent-(+)-**amorpha-4,11-diene** (8), (-)-amorpha-4,7(11)-diene (9), the sesquiterpene alcohol (+)-9-hydroxyselina-4,11-diene (10) and (-)-2-acetoxyamorpha-4,7(11)-diene (11) were identified. In M. alpina (-)-trans-selina-4(15),11-dien-5-ol (12), (+)-8,9-epoxyselina-4,11-diene (13) and (+)-cis-selina-4(15),11-dien-5-ol (14) were found as new natural products.

L5 ANSWER 9 OF 15 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001197498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11289612
TITLE: **Amorpha-4,11-diene** synthase: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin.
AUTHOR: Wallaart T E; Bouwmeester H J; Hille J; Poppinga L; Maijers N C
CORPORATE SOURCE: GenoClipp Biotechnology BV, Meditech Center, Groningen, The Netherlands.. mail@genoclipp.com
SOURCE: Planta, (2001 Feb) 212 (3) 460-5.
Journal code: 1250576. ISSN: 0032-0935.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY006482
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719
AB The sesquiterpenoid artemisinin, isolated these from the plant Artemisia annua L., and its semi-synthetic derivatives are a new and very effective group of antimalarial drugs. A branch point in the biosynthesis of this compound is the cyclisation of the ubiquitous precursor farnesyl diphosphate into the first specific precursor of artemisinin, namely **amorpha-4,11-diene**. Here we describe the isolation of a cDNA clone encoding **amorpha-4,11-diene** synthase. The deduced amino acid sequence exhibits the highest identity (50%) with a putative sesquiterpene cyclase of A. annua. When expressed in Escherichia coli, the recombinant enzyme catalyses the formation of **amorpha-4,11-diene** from farnesyl diphosphate. Introduction of the gene into tobacco (Nicotiana tabacum L.) resulted in the expression of an active enzyme and the accumulation of **amorpha-4,11-diene** ranging from 0.2 to 1.7 ng per g fresh weight.

L5 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:314515 HCAPLUS
DOCUMENT NUMBER: 135:134657
TITLE: Volatile constituents in mosses (Musci)
AUTHOR(S): Saritas, Y.; Sonwa, M. M.; Iznaguen, H.; Konig, W. A.; Muhle, H.; Mues, R.
CORPORATE SOURCE: Institut fur Organische Chemie, Universitat Hamburg, Hamburg, D-20146, Germany
SOURCE: Phytochemistry (2001), 57(3), 443-457
CODEN: PYTCAS; ISSN: 0031-9422

PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The essential oils of mosses of the genera Mnium, Plagiomnium, Homalia, Plagiothecium and Taxiphyllum (Musci) have been investigated by gas chromatog. and mass spectrometry. The new sesquiterpenes (+)-10-epi-muurola-4,11-diene (I) and 10,11-dihydro-.alpha.-cuparenone (II) were isolated by preparative gas chromatog. and identified as major constituents of the hydrodistn. products of Mnium hornum (Hedw.) using NMR and mass spectrometry. In addn., (+)-dauca-8,11-diene (III) and two new butenolides, 3,4,5-trimethyl-5-pentyl-5H-furan-2-one and 3,4-dimethyl-5-pentyl-5H-furan-2-one were identified as constituents in Plagiomnium undulatum (Hedw.) T. Kop. Although the amts. of volatiles present in the investigated mosses are generally smaller than in liverworts, the spectrum of terpenoid compds. is similar. The investigated mosses also generate aliph. compds. of greater abundance and structural variety.
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 11 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:144616 HCAPLUS
DOCUMENT NUMBER: 132:204840
TITLE: Artemisia annua **amorpha-4,11-diene** synthase, its cDNA, recombinant expression, and methods of **amorpha-4,11-diene** and artemisinin synthesis via transgenic plants
INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan
PATENT ASSIGNEE(S): Neth.
SOURCE: Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 982404	A1	20000301	EP 1998-202854	19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2340925	AA	20000309	CA 1999-2340925	19990827
WO 2000012725	A2	20000309	WO 1999-EP6302	19990827
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9957423	A1	20000321	AU 1999-57423	19990827
AU 766764	B2	20031023		
EP 1108041	A2	20010620	EP 1999-944535	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 9913196	A	20010925	BR 1999-13196	19990827
JP 2002523101	T2	20020730	JP 2000-567711	19990827
ZA 2001001455	A	20010828	ZA 2001-1455	20010221

PRIORITY APPLN. INFO.: EP 1998-202854 A 19980827
WO 1999-EP6302 W 19990827

AB **Amorpha-4,11-diene** synthase from Artemisia annua L., its cDNA, recombinant expression, and methods of

prepg. **amorpha-4,11-diene** and
artemisinin from farnesyl pyrophosphate (FPP) using transgenic organism are provided. **Amorpha-4,11-diene** is a precursor of the new anti-malarial drug artemisinin produced by the plant Artemisia annua L. A cDNA encoding **amorpha-4, 11-diene** synthase from A. annua has been isolated and sequenced, and the corresponding amino acid sequence has been detd. Recombinant **amorpha-4,11-diene** synthase expressed in E. coli, transgenic tobacco, and transgenic A. annua catalyzed conversion of FPP into **amorpha-4,11 -diene**. Further conversion of **amorpha-4, 11-diene** into artemisinin was obsd. in transgenic A. annua. The invention may be useful in obtaining enhanced prodn. of stereochem. desirable artemisinin.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 12 OF 15 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001128077 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11185551
TITLE: **Amorpha-4,11-diene** synthase of Artemisia annua: cDNA isolation and bacterial expression of a terpene synthase involved in artemisinin biosynthesis.
AUTHOR: Chang Y J; Song S H; Park S H; Kim S U
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, Korea.
SOURCE: Archives of biochemistry and biophysics, (2000 Nov 15) 383 (2) 178-84.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ251751
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express **amorpha-4 ,11-diene** synthase for metabolic engineering. PCR amplification of genomic DNA with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp DNA fragment. This fragment was used in Northern blot analysis as a probe, showing approximately 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A soluble fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS analysis as **amorpha-4,11-diene**.

L5 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2000479808 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11032404
TITLE: Molecular cloning, expression, and characterization of **amorpha-4,11-diene** synthase, a key enzyme of artemisinin biosynthesis in

Artemisia annua L.
AUTHOR: Mercke P; Bengtsson M; Bouwmeester H J; Posthumus M A; Brodelius P E
CORPORATE SOURCE: Department of Plant Biochemistry, Lund University, Sweden.
SOURCE: Archives of biochemistry and biophysics, (2000 Sep 15) 381 (2) 173-80.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF138959
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001031

AB In plants, sesquiterpenes of different structural types are biosynthesized from the isoprenoid intermediate farnesyl diphosphate. The initial reaction of the biosynthesis is catalyzed by sesquiterpene cyclases (synthases). In Artemisia annua L. (annual wormwood), a number of such sesquiterpene cyclases are active. We have isolated a cDNA clone encoding one of these, **amorpha-4,11-diene** synthase, a putative key enzyme of artemisinin biosynthesis. This clone contains a 1641-bp open reading frame coding for 546 amino acids (63.9 kDa), a 12-bp 5'-untranslated end, and a 427-bp 3'-untranslated sequence. The deduced amino acid sequence is 32 to 51% identical with the sequence of other known sesquiterpene cyclases from angiosperms. When expressed in Escherichia coli, the recombinant enzyme catalyzed the formation of both olefinic (97.5%) and oxygenated (2.5%) sesquiterpenes from farnesyl diphosphate. GC-MS analysis identified the olefins as (E)-beta-farnesene (0.8%), amorpha-4,11diene (91.2%), amorpha-4,7(11)-diene (3.7%), gamma-humulene (1.0%), beta-sesquiphellandrene (0.5%), and an unknown olefin (0.2%) and the oxygenated sesquiterpenes as amorpha-4-en-11-ol (0.2%) (tentatively), amorpha-4-en-7-ol (2.1%), and alpha-bisabolol (0.3%) (tentatively). Using geranyl diphosphate as substrate, **amorpha-4,11-diene** synthase did not produce any monoterpenes. The recombinant enzyme has a broad pH optimum between 7.5 and 9.0 and the Km values for farnesyl diphosphate, Mg2+, and Mn2+ are 0.9, 70, and 13 microM, respectively, at pH 7.5. A putative reaction mechanism for **amorpha-4,11-diene** synthase is suggested.

L5 ANSWER 14 OF 15 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2000091820 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10626375
TITLE: **Amorpha-4,11-diene** synthase catalyses the first probable step in artemisinin biosynthesis.
AUTHOR: Bouwmeester H J; Wallaart T E; Janssen M H; van Loo B; Jansen B J; Posthumus M A; Schmidt C O; De Kraker J W; Konig W A; Franssen M C
CORPORATE SOURCE: Research Institute for Agrobiology and Soil Fertility (AB-DLO), Wageningen, Netherlands..
h.j.bouwmeester@ab.dlo.nl
SOURCE: Phytochemistry, (1999 Nov) 52 (5) 843-54.
Journal code: 0151434. ISSN: 0031-9422.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000211

AB The endoperoxide sesquiterpene lactone artemisinin and its derivatives are

a promising new group of drugs against malaria. Artemisinin is a constituent of the annual herb Artemisia annua L. So far only the later steps in artemisinin biosynthesis--from artemisinic acid--have been elucidated and the expected olefinic sesquiterpene intermediate has never been demonstrated. In pentane extracts of A. annua leaves we detected a sesquiterpene with the mass spectrum of **amorpha-4, 11-diene**. Synthesis of **amorpha-4, 11-diene** from artemisinic acid confirmed the identity. In addition we identified several sesquiterpene synthases of which one of the major activities catalysed the formation of **amorpha-4,11-diene** from farnesyl diphosphate. This enzyme was partially purified and shows the typical characteristics of sesquiterpene synthases, such as a broad pH optimum around 6.5-7.0, a molecular mass of 56 kDa, and a K(m) of 0.6 microM. The structure and configuration of **amorpha-4,11-diene**, its low content in A. annua and the high activity of **amorpha-4,11-diene** synthase all support that **amorpha-4,11-diene** is the likely olefinic sesquiterpene intermediate in the biosynthesis of artemisinin.

L5 ANSWER 15 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 2000:112831 HCAPLUS
DOCUMENT NUMBER: 132:305749
TITLE: Constituents of the leaf essential oil of Cedrela odorata L. from Nigeria
AUTHOR(S): Asekun, O. T.; Ekundayo, O.
CORPORATE SOURCE: Department of Chemistry, University of Ibadan, Ibadan, Nigeria
SOURCE: Flavour and Fragrance Journal (1999), 14(6), 390-392
CODEN: FFJOED; ISSN: 0882-5734
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The essential oil compn. of Cedrela odorata L. leaves was comprehensively investigated by means of capillary GC and GC-MS. Twenty-six constituents were identified in the volatile oil. Sesquiterpenoids such as .alpha.-santalene (9.5%), .beta.-acoradiene (7.1%), .beta.-elemene (6.8%), caryophyllene oxide (6.0%) and Z-.alpha.-bergamotene (6.0%) were the dominant compds. Minor constituents included isocaryophyllene, .beta.-bisabolene, .beta.-alaskene and **amorpha-4, 11-diene**. A rare sesquiterpenoid sulfur deriv., mintsulfide, was identified for the first time in C. odorata essential oil.
REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 15:10:54 ON 08 JUN 2004)

FILE 'MEDLINE, HCAPLUS, EMBASE' ENTERED AT 15:11:16 ON 08 JUN 2004
L1 8 S AMORPHADIENE AND SYNTHASE
L2 6 DUP REM L1 (2 DUPLICATES REMOVED)
L3 1 S AMORPHADIENE AND DNA
L4 25 S AMORPHA-4,11-DIENE
L5 15 DUP REM L4 (10 DUPLICATES REMOVED)

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FULL ESTIMATED COST	47.23	47.44

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION

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STN INTERNATIONAL LOGOFF AT 15:15:14 ON 08 JUN 2004

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L2: Entry 1 of 1 File: PGPB Jan 8, 2004

PGPUB-DOCUMENT-NUMBER: 20040005678
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040005678 A1

TITLE: Biosynthesis of amorpha-4,11-diene

PUBLICATION-DATE: January 8, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Keasling, Jay	Berkeley	CA	US	
Martin, Vincent	Kensington	CA	US	
Pitera, Douglas	Oakland	CA	US	
Withers, Sydnor T. III	Richmond	CA	US	
Newman, Jack	Berkeley	CA	US	

APPL-NO: 10/ 411066 [PALM]
DATE FILED: April 9, 2003

RELATED-US-APPL-DATA:
Application 10/411066 is a continuation-in-part-of US application 10/006909, filed December 6, 2001, PENDING

INT-CL: [07] C12 P 7/42, C12 N 9/10, C07 H 21/04, C12 N 1/21, C12 N 15/74

US-CL-PUBLISHED: 435/146; 435/193, 435/252.3, 435/320.1, 536/23.2
US-CL-CURRENT: 435/146; 435/193, 435/252.3, 435/320.1, 536/23.2

REPRESENTATIVE-FIGURES: 1A

ABSTRACT:

Methods for synthesizing amorpha-4,11-diene synthase from isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. Amorpha-4,11-diene synthase is then produced using an optimized amorpha-4,11-diene synthase gene. The invention also provides nucleic acid sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a continuation-in-part of U.S. patent application Ser. No. 10/006,909, filed on Dec. 6, 2001, the disclosure of which is incorporated by

reference in its entirety.

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=> file medline caplus biosis biotechds embase scisearch
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.42       0.42

FILE 'MEDLINE' ENTERED AT 14:53:07 ON 08 JUN 2004

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=> s (amorphadiene synthase or amorpha-4 11-diene synthase)
L1          35 (AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE)

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          13 DUP REM L1 (22 DUPLICATES REMOVED)

=> d l2 1-3 ibib ab

L2   ANSWER 1 OF 13  CAPLUS  COPYRIGHT 2004 ACS on STN DUPLICATE 1
ACCESSION NUMBER:        2004:18837  CAPLUS
DOCUMENT NUMBER:         140:92683
TITLE:                   Preparation of amorpha-4,11-diene with transgenic
                         microorganisms producing isopentenyl- and
                         dimethylallyl pyrophosphates
INVENTOR(S):             Keasling, Jay; Martin, Vincent; Pitera, Douglas;
                         Withers, Sydnor T.; Newman, Jack
PATENT ASSIGNEE(S):      USA
SOURCE:                  U.S. Pat. Appl. Publ., 75 pp., Cont.-in-part of U.S.
                         Ser. No. 6,909.
                         CODEN: USXXCO
DOCUMENT TYPE:           Patent
LANGUAGE:                English
FAMILY ACC. NUM. COUNT:  2
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     --------------    ----  --------      ---------------  --------
     US 2004005678      A1   20040108      US 2003-411066   20030409
     US 2003148479      A1   20030807      US 2001-6909     20011206
PRIORITY APPLN. INFO.:                  US 2001-6909     A2 20011206
AB   Methods for synthesizing amorpha-4,11-diene from isopentenyl pyrophosphate
     are provided.  A first method comprises introducing into a host
     microorganism a plurality of heterologous nucleic acid sequences, each
     coding for a different enzyme in the mevalonate pathway for producing
     isopentenyl pyrophosphate.  Amorpha-4,11-diene is then produced with the
     transgenic microorganism which is further transformed with an optimized
     amorpha-4,11-diene synthase
     gene.  The amorpha-4,11-diene may be used in synthesis of the antimalarial
     drug artemisinin.  Thus, amorpha-4,11-diene was prepd. from mevalonate
     supplied in the medium with Escherichia coli transformed with plasmid
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pBBRMDIS-2, contg. the yeast genes idi (for isopentenyl pyrophosphate isomerase) and ispA (for farnesyl pyrophosphate synthase) and the genes for mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, and **amorpha-4,11-diene synthase**. The yield was 2 .mu.g amorpha-4,11-diene/mL.

L2 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:609986 CAPLUS
DOCUMENT NUMBER: 139:160786
TITLE: Biosynthesis of isopentenyl pyrophosphate using recombinant microbial metabolic pathways
INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim, Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo; Newman, Jack; Khlebnikov, Artem Valentinovich
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 40 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003148479	A1	20030807	US 2001-6909	20011206
US 2004005678	A1	20040108	US 2003-411066	20030409

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206
AB Methods for synthesizing isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. A related method comprises introducing into a host microorganism an intermediate in the mevalonate pathway and at least one heterologous nucleic acid sequence, each sequence coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into isopentenyl pyrophosphate. The invention also provides nucleic acid sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

L2 ANSWER 3 OF 13 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003324605 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12778056
TITLE: Engineering a mevalonate pathway in Escherichia coli for production of terpenoids.
AUTHOR: Martin Vincent J J; Pitera Douglas J; Withers Sydnor T; Newman Jack D; Keasling Jay D
CORPORATE SOURCE: Department of Chemical Engineering, 201 Gilman Hall, University of California, Berkeley, California 94720-1462, USA.
SOURCE: Nature biotechnology, (2003 Jul) 21 (7) 796-802.
Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200404
ENTRY DATE: Entered STN: 20030713
Last Updated on STN: 20040407
Entered Medline: 20040406
AB Isoprenoids are the most numerous and structurally diverse family of natural products. Terpenoids, a class of isoprenoids often isolated from plants, are used as commercial flavor and fragrance compounds and antimalarial or anticancer drugs. Because plant tissue extractions typically yield low terpenoid concentrations, we sought an alternative

method to produce high-value terpenoid compounds, such as the antimalarial drug artemisinin, in a microbial host. We engineered the expression of a synthetic **amorpha-4,11-diene synthase** gene and the mevalonate isoprenoid pathway from Saccharomyces cerevisiae in Escherichia coli. Concentrations of amorphadiene, the sesquiterpene olefin precursor to artemisinin, reached 24 microg caryophyllene equivalent/ml. Because isopentenyl and dimethylallyl pyrophosphates are the universal precursors to all isoprenoids, the strains developed in this study can serve as platform hosts for the production of any terpenoid compound for which a terpene synthase gene is available.

=> d l2 4-13 ibib ab

L2 ANSWER 4 OF 13 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2003:687697 SCISEARCH
THE GENUINE ARTICLE: 708AQ
TITLE: Scale-up of Artemisia annua L. hairy root cultures produces complex patterns of terpenoid gene expression
AUTHOR: Souret F F; Kim Y; Wysiouzil B E; Wobbe K K; Weathers P J (Reprint)
CORPORATE SOURCE: Worcester Polytech Inst, Dept Biol & Biotechnol, Worcester, MA 01609 USA (Reprint); Worcester Polytech Inst, Dept Chem Engn, Worcester, MA 01609 USA; Worcester Polytech Inst, Dept Chem & Biochem, Worcester, MA 01609 USA
COUNTRY OF AUTHOR: USA
SOURCE: BIOTECHNOLOGY AND BIOENGINEERING, (20 SEP 2003) Vol. 83, No. 6, pp. 653-667.
Publisher: JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA.
ISSN: 0006-3592.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 86

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Hairy roots grow quickly, reach high densities, and can produce significant amounts of secondary metabolites, yet their scale-up to bioreactors remains challenging. Artemisia annua produces a rich array of terpenoids, including the sesquiterpene, artemisinin, and transformed roots of this species provide a good model for studying terpenoid production. These cultures were examined in shake flasks and compared with cultures grown in two types of bioreactors, a mist reactor and a bubble column reactor, which provide very different environments for the growing roots. Mist reactors have been shown previously to result in cultures that produce significantly more artemisinin per gram fresh weight of culture, while bubble column reactors have produced greater biomass. We have compared expression levels of four key terpenoid biosynthetic genes: 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), 1-deoxy-D-xylulose-5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), and farnesyl diphosphate synthase (FPS) in the three culture conditions. In shake flasks we found that although all four genes showed temporal regulation, only FPS expression correlated with artemisinin production. Light also affected the transcription of all four genes. Although expression in reactors was equivalent to or greater than that of roots grown in shake flasks, no correlation was found between expression level within six different zones of each reactor and their respective oxygen levels, light, and root-packing density. Surprisingly, transcriptional regulation of HMGR, DXS, DXR, and FPS was greatly affected by the position of the roots in each reactor. Thus, relying on a single reactor sample to characterize the gene activity in a whole reactor can be misleading, especially if the goal is to examine the difference between reactor types or operating parameters, steps essential in scaling up cultures for production. (C) 2003 Wiley Periodicals, Inc.

L2 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2004:187822 CAPLUS
TITLE: Cloning, E. coli expression and molecular analysis of a novel sesquiterpene synthase gene from Artemisia annua
AUTHOR(S): Liu, Yan; Ye, Hechun; Li, Guofeng
CORPORATE SOURCE: Key laboratory of Plant Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, Peop. Rep. China
SOURCE: Zhiwu Xuebao (2002), 44(12), 1450-1455
CODEN: CHWHAY; ISSN: 0577-7496
PUBLISHER: Kexue Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 1 886 bp full-length sesquiterpene synthase (AaSES) cDNA was cloned from a high-yield Artemisia annua L. strain 001 by a rapid amplification of cDNA end (RACE) strategy. AaSES was 59% identical to Artemisia cyclase cDNA clone cASC125, 50% identical to epi-cedrol synthase from A. annua, 48% identical to **amorpha-4,11-diene synthase** from A. annua, 39% identical to the 5-epi-aristolechene synthase from tobacco, 38% identical to vetispiradiene synthase from H. muticus, 41 % identical to the .delta.-cadinene synthase from cotton. The coding region of the cDNA was inserted into a procaryotic expression vector pET-30a and overexpressed in E. coli BL21 (DE3). The cyclase proteins extd. from bacterial culture were found largely in an insol. protein fraction. AaSES expressed in leaves, stems and flowers, not in roots as indicated by Northern blotting anal.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 6 OF 13 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:972593 SCISEARCH
THE GENUINE ARTICLE: 619KZ
TITLE: A cDNA clone for beta-caryophyllene synthase from Artemisia annua
AUTHOR: Cai Y; Jia J W; Crock J; Lin Z X; Chen X Y; Croteau R (Reprint)
CORPORATE SOURCE: Washington State Univ, Inst Biol Chem, Pullman, WA 99164 USA (Reprint); Chinese Acad Sci, Shanghai Inst Biol Sci, Inst Plant Physiol & Ecol, Natl Lab Plant Mol Genet, Shanghai 200032, Peoples R China; Shanghai Jiao Tong Univ, Coll Life Sci & Biotechnol, Shanghai 200030, Peoples R China
COUNTRY OF AUTHOR: USA; Peoples R China
SOURCE: PHYTOCHEMISTRY, (NOV 2002) Vol. 61, No. 5, pp. 523-529. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0031-9422.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 34
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB An homology-based cloning strategy yielded a full-length cDNA from Artemisia annua that encoded a protein of 60.3 kDa which resembled a sesquiterpene synthase in sequence. Heterologous expression of the gene in Escherichia coli provided a soluble recombinant enzyme capable of catalyzing the divalent metal ion-dependent conversion of farnesyl diphosphate to beta-caryophyllene, a sesquiterpene olefin found in the essential oil of A. annua. In reaction parameters and kinetic properties, beta-caryophyllene synthase resembles other sesquiterpene synthases of angiosperms. The beta-caryophyllene synthase gene is expressed in most plant tissues during early development, and is induced in mature tissue in response to fungal elicitor thus suggesting a role for beta-caryophyllene

in plant defense. (C) 2002 Elsevier Science Ltd. All rights reserved.

L2 ANSWER 7 OF 13 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:780812 SCISEARCH
THE GENUINE ARTICLE: 595BV
TITLE: Cloning and functional characterization of a beta-pinene synthase from Artemisia annua that shows a circadian pattern of expression
AUTHOR: Lu S; Xu R; Jia J W; Pang J H; Matsuda S P T; Chen X Y (Reprint)
CORPORATE SOURCE: Chinese Acad Sci, Shanghai Inst Biol Sci, Inst Plant Physiol & Ecol, Natl Lab Plant Mol Genet, Shanghai 200032, Peoples R China (Reprint); Rice Univ, Dept Chem, Houston, TX 77251 USA; Rice Univ, Dept Biochem & Cell Biol, Houston, TX 77251 USA
COUNTRY OF AUTHOR: Peoples R China; USA
SOURCE: PLANT PHYSIOLOGY, (SEP 2002) Vol. 130, No. 1, pp. 477-486. Publisher: AMER SOC PLANT BIOLOGISTS, 15501 MONONA DRIVE, ROCKVILLE, MD 20855 USA. ISSN: 0032-0889.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Artemisia annua plants produce a broad range of volatile compounds, including monoterpenes, which contribute to the characteristic fragrance of this medicinal species. A cDNA clone, QH6, contained an open reading frame encoding a 582-amino acid protein that showed high sequence identity to plant monoterpene synthases. The prokaryotically expressed QH6 fusion protein converted geranyl diphosphate to (-)-beta-pinene and (-)-alpha-pinene in a 94:6 ratio. QH6 was predominantly expressed in juvenile leaves 2 weeks postsprouting. QH6 transcript levels were transiently reduced following mechanical wounding or fungal elicitor treatment, suggesting that this gene is not directly involved in defense reaction induced by either of these treatments. Under a photoperiod of 12 h/12 h (light/dark), the abundance of QH6 transcripts fluctuated in a diurnal pattern that ebbed around 3 h before daybreak (9th h in the dark phase) and peaked after 9 h in light (9th h in the light phase). The contents of (-)-beta-pinene in juvenile leaves and in emitted volatiles also varied in a diurnal rhythm, correlating strongly with mRNA accumulation. When A. annua was entrained by constant light or constant dark conditions, QH6 transcript accumulation continued to fluctuate with circadian rhythms. Under constant light, advanced cycles of fluctuation of QH6 transcript levels were observed, and under constant dark, the cycle was delayed. However, the original diurnal pattern could be regained when the plants were returned to the normal light/dark (12 h/12 h) photoperiod. This is the first report that monoterpene biosynthesis is transcriptionally regulated in a circadian pattern.

L2 ANSWER 8 OF 13 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:467930 SCISEARCH
THE GENUINE ARTICLE: 554XG
TITLE: Isolation and characterization of two germacrene A synthase cDNA clones from chicory
AUTHOR: Bouwmeester H J (Reprint); Kodde J; Verstappen F W A; Altug I G; de Kraker J W; Wallaart T E
CORPORATE SOURCE: Plant Res Int, Business Unit Cell Cybernet, POB 16, NL-6700 AA Wageningen, Netherlands (Reprint); Plant Res Int, Business Unit Cell Cybernet, NL-6700 AA Wageningen, Netherlands; Univ Hamburg, Dept Organ Chem, D-20146 Hamburg, Germany; Wageningen Univ Agr, Dept Organ Chem, NL-6703 HB Wageningen, Netherlands; Univ Groningen, Univ Ctr Pharm, Dept Pharmaceut Biol, NL-9713 AV Groningen, Netherlands
COUNTRY OF AUTHOR: Netherlands; Germany

SOURCE: PLANT PHYSIOLOGY, (MAY 2002) Vol. 129, No. 1, pp. 134-144.
Publisher: AMER SOC PLANT BIOLOGISTS, 15501 MONONA DRIVE, ROCKVILLE, MD 20855 USA.
ISSN: 0032-0889.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 29

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chicory (Cichorium intybus) sesquiterpene lactones were recently shown to be derived from a common sesquiterpene intermediate, (+)-germacrene A. Germacrene A is of interest because of its key role in sesquiterpene lactone biosynthesis and because it is an enzyme-bound intermediate in the biosynthesis of a number of phytoalexins. Using polymerase chain reaction with degenerate primers, we have isolated two sesquiterpene synthases from chicory that exhibited 72% amino acid identity. Heterologous expression of the genes in Escherichia coli has shown that they both catalyze exclusively the formation of (+)-germacrene A, making this the first report, to our knowledge, on the isolation of (+)-germacrene A synthase (GAS)-encoding genes. Northern analysis demonstrated that both genes were expressed in all chicory tissues tested albeit at varying levels. Protein isolation and partial purification from chicory heads demonstrated the presence of two GAS proteins. On MonoQ, these proteins co-eluted with the two heterologously produced proteins. The K-m value, pH optimum, and MonoQ elution volume of one of the proteins produced in E. coli were similar to the values reported for the GAS protein that was recently purified from chicory roots. Finally, the two deduced amino acid sequences were modeled, and the resulting protein models were compared with the crystal structure of tobacco (Nicotiana tabacum) 5-epi-aristolochene synthase, which forms germacrene A as an enzyme-bound intermediate en route to 5-epi-aristolochene. The possible involvement of a number of amino acids in sesquiterpene synthase product specificity is discussed.

L2 ANSWER 9 OF 13 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001197498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11289612
TITLE: **Amorpha-4,11-diene synthase**: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin.
AUTHOR: Wallaart T E; Bouwmeester H J; Hille J; Poppinga L; Maijers N C
CORPORATE SOURCE: GenoClipp Biotechnology BV, Meditech Center, Groningen, The Netherlands.. mail@genoclipp.com
SOURCE: Planta, (2001 Feb) 212 (3) 460-5.
Journal code: 1250576. ISSN: 0032-0935.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY006482
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719

AB The sesquiterpenoid artemisinin, isolated these from the plant Artemisia annua L., and its semi-synthetic derivatives are a new and very effective group of antimalarial drugs. A branch point in the biosynthesis of this compound is the cyclisation of the ubiquitous precursor farnesyl diphosphate into the first specific precursor of artemisinin, namely amorpha-4,11-diene. Here we describe the isolation of a cDNA clone encoding **amorpha-4,11-diene synthase**. The deduced amino acid sequence exhibits the highest identity (50%) with a putative sesquiterpene cyclase of A. annua. When expressed in Escherichia coli, the recombinant enzyme catalyses the formation of amorpha-4,11-diene from farnesyl diphosphate. Introduction

of the gene into tobacco (Nicotiana tabacum L.) resulted in the expression of an active enzyme and the accumulation of amorpha-4,11-diene ranging from 0.2 to 1.7 ng per g fresh weight.

L2 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:144616 CAPLUS
DOCUMENT NUMBER: 132:204840
TITLE: Artemisia annua **amorpha-4, 11-diene synthase**, its cDNA, recombinant expression, and methods of amorpha-4,11-diene and artemisinin synthesis via transgenic plants
INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan
PATENT ASSIGNEE(S): Neth.
SOURCE: Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 982404	A1	20000301	EP 1998-202854	19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2340925	AA	20000309	CA 1999-2340925	19990827
WO 2000012725	A2	20000309	WO 1999-EP6302	19990827
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9957423	A1	20000321	AU 1999-57423	19990827
AU 766764	B2	20031023		
EP 1108041	A2	20010620	EP 1999-944535	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 9913196	A	20010925	BR 1999-13196	19990827
JP 2002523101	T2	20020730	JP 2000-567711	19990827
ZA 2001001455	A	20010828	ZA 2001-1455	20010221

PRIORITY APPLN. INFO.: EP 1998-202854 A 19980827
WO 1999-EP6302 W 19990827

AB **Amorpha-4,11-diene synthase** from Artemisia annua L., its cDNA, recombinant expression, and methods of prepg. amorpha-4,11-diene and artemisinin from farnesyl pyrophosphate (FPP) using transgenic organism are provided. Amorpha-4,11-diene is a precursor of the new anti-malarial drug artemisinin produced by the plant Artemisia annua L. A cDNA encoding **amorpha-4,11-diene synthase** from A. annua has been isolated and sequenced, and the corresponding amino acid sequence has been detd. Recombinant **amorpha-4, 11-diene synthase** expressed in E. coli, transgenic tobacco, and transgenic A. annua catalyzed conversion of FPP into amorpha-4,11-diene. Further conversion of amorpha-4,11-diene into artemisinin was obsd. in transgenic A. annua. The invention may be useful in obtaining enhanced prodn. of stereochem. desirable artemisinin.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 11 OF 13 MEDLINE on STN DUPLICATE 5

ACCESSION NUMBER: 2001128077 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11185551
TITLE: **Amorpha-4,11-diene synthase** of Artemisia annua: cDNA isolation and bacterial expression of a terpene synthase involved in artemisinin biosynthesis.
AUTHOR: Chang Y J; Song S H; Park S H; Kim S U
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, Korea.
SOURCE: Archives of biochemistry and biophysics, (2000 Nov 15) 383 (2) 178-84.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ251751
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express **amorpha-4,11-diene synthase** for metabolic engineering. PCR amplification of genomic DNA with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp DNA fragment. This fragment was used in Northern blot analysis as a probe, showing approximately 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A soluble fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS analysis as amorpha-4,11-diene.

L2 ANSWER 12 OF 13 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2000479808 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11032404
TITLE: Molecular cloning, expression, and characterization of **amorpha-4,11-diene synthase**, a key enzyme of artemisinin biosynthesis in Artemisia annua L.
AUTHOR: Mercke P; Bengtsson M; Bouwmeester H J; Posthumus M A; Brodelius P E
CORPORATE SOURCE: Department of Plant Biochemistry, Lund University, Sweden.
SOURCE: Archives of biochemistry and biophysics, (2000 Sep 15) 381 (2) 173-80.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF138959
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001031

AB In plants, sesquiterpenes of different structural types are biosynthesized from the isoprenoid intermediate farnesyl diphosphate. The initial

reaction of the biosynthesis is catalyzed by sesquiterpene cyclases (synthases). In Artemisia annua L. (annual wormwood), a number of such sesquiterpene cyclases are active. We have isolated a cDNA clone encoding one of these, **amorpha-4,11-diene synthase**, a putative key enzyme of artemisinin biosynthesis. This clone contains a 1641-bp open reading frame coding for 546 amino acids (63.9 kDa), a 12-bp 5'-untranslated end, and a 427-bp 3'-untranslated sequence. The deduced amino acid sequence is 32 to 51% identical with the sequence of other known sesquiterpene cyclases from angiosperms. When expressed in Escherichia coli, the recombinant enzyme catalyzed the formation of both olefinic (97.5%) and oxygenated (2.5%) sesquiterpenes from farnesyl diphosphate. GC-MS analysis identified the olefins as (E)-beta-farnesene (0.8%), amorpha-4,11diene (91.2%), amorpha-4,7(11)-diene (3.7%), gamma-humulene (1.0%), beta-sesquiphellandrene (0.5%), and an unknown olefin (0.2%) and the oxygenated sesquiterpenes as amorpha-4-en-11-ol (0.2%) (tentatively), amorpha-4-en-7-ol (2.1%), and alpha-bisabolol (0.3%) (tentatively). Using geranyl diphosphate as substrate, **amorpha-4,11-diene synthase** did not produce any monoterpenes. The recombinant enzyme has a broad pH optimum between 7.5 and 9.0 and the Km values for farnesyl diphosphate, Mg2+, and Mn2+ are 0.9, 70, and 13 microM, respectively, at pH 7.5. A putative reaction mechanism for **amorpha-4,11-diene synthase** is suggested.

L2 ANSWER 13 OF 13 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 2000091820 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10626375
TITLE: **Amorpha-4,11-diene synthase** catalyses the first probable step in artemisinin biosynthesis.
AUTHOR: Bouwmeester H J; Wallaart T E; Janssen M H; van Loo B; Jansen B J; Posthumus M A; Schmidt C O; De Kraker J W; Konig W A; Franssen M C
CORPORATE SOURCE: Research Institute for Agrobiology and Soil Fertility (AB-DLO), Wageningen, Netherlands..
h.j.bouwmeester@ab.dlo.nl
SOURCE: Phytochemistry, (1999 Nov) 52 (5) 843-54.
Journal code: 0151434. ISSN: 0031-9422.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000211

AB The endoperoxide sesquiterpene lactone artemisinin and its derivatives are a promising new group of drugs against malaria. Artemisinin is a constituent of the annual herb Artemisia annua L. So far only the later steps in artemisinin biosynthesis--from artemisinic acid--have been elucidated and the expected olefinic sesquiterpene intermediate has never been demonstrated. In pentane extracts of A. annua leaves we detected a sesquiterpene with the mass spectrum of amorpha-4,11-diene. Synthesis of amorpha-4,11-diene from artemisinic acid confirmed the identity. In addition we identified several sesquiterpene synthases of which one of the major activities catalysed the formation of amorpha-4,11-diene from farnesyl diphosphate. This enzyme was partially purified and shows the typical characteristics of sesquiterpene synthases, such as a broad pH optimum around 6.5-7.0, a molecular mass of 56 kDa, and a K(m) of 0.6 microM. The structure and configuration of amorpha-4,11-diene, its low content in A. annua and the high activity of **amorpha-4,11-diene synthase** all support that amorpha-4,11-diene is the likely olefinic sesquiterpene intermediate in the biosynthesis of artemisinin.

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                                                          ENTRY    SESSION
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                                                          ENTRY    SESSION
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DICTIONARY FILE UPDATES:   7 JUN 2004  HIGHEST RN 690625-61-7

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=> s AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE
             1 AMORPHADIENE
         25230 SYNTHASE
             0 AMORPHADIENE SYNTHASE
                 (AMORPHADIENE(W)SYNTHASE)
            28 AMORPHA
      13754855 4
        873305 11
        210060 DIENE
         25230 SYNTHASE
             6 AMORPHA-4 11-DIENE SYNTHASE
                 (AMORPHA(W)4(W)11(W)DIENE(W)SYNTHASE)
L3           6 AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE

=> d l3 1-6

L3   ANSWER 1 OF 6  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   642550-56-9  REGISTRY
CN   DNA (synthetic Saccharomyces cerevisiae amorpha-4,11-diene synthase
     gene) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   37: PN: US20040005678 SEQID: 37 claimed DNA
FS   NUCLEIC ACID SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
DT.CA  CAplus document type:  Patent
RL.P   Roles from patents:  BIOL (Biological study); PRP (Properties); USES
       (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
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1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3 ANSWER 2 OF 6 REGISTRY COPYRIGHT 2004 ACS on STN
RN 337549-56-1 REGISTRY
CN Synthase, amorpha-4,11-diene (Artemisia annua) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Amorpha-4,11-diene synthase (Artemisia annua)**
CN GenBank AAF98444
CN GenBank AAF98444 (Translated from: GenBank AY006482)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3 ANSWER 3 OF 6 REGISTRY COPYRIGHT 2004 ACS on STN
RN 286830-30-6 REGISTRY
CN **DNA (Artemisia annua amorpha-4,11-diene synthase cDNA plus flanks) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AY006482
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: BIOSIS, CA, CAPLUS, GENBANK
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3 ANSWER 4 OF 6 REGISTRY COPYRIGHT 2004 ACS on STN
RN 271555-04-5 REGISTRY
CN **DNA (Artemisia annua strain South-Korea/Suwon gene kcs12 amorpha-4,11-diene synthase cDNA plus flanks) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AJ251751
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS, GENBANK
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3 ANSWER 5 OF 6 REGISTRY COPYRIGHT 2004 ACS on STN
RN 260231-31-0 REGISTRY
CN **DNA (Artemisia annua amorpha-4,11-diene synthase cDNA) (9CI)**

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     (CA INDEX NAME)
OTHER NAMES:
CN   21: PN: EP982404 FIGURE: 9 claimed DNA
FS   NUCLEIC ACID SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS
DT.CA  CAplus document type:  Patent
RL.P   Roles from patents:  BIOL (Biological study); PROC (Process); PRP
       (Properties); USES (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3   ANSWER 6 OF 6  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   259213-60-0  REGISTRY
CN   Synthase, amorpha-4,11-diene (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Amorpha-4,11-diene synthase
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   BIOSIS, CA, CAPLUS, TOXCENTER, USPATFULL
DT.CA  CAplus document type:  Journal; Patent
RL.P   Roles from patents:  BIOL (Biological study); OCCU (Occurrence); PREP
       (Preparation); PROC (Process); PRP (Properties); USES (Uses)
RL.NP  Roles from non-patents:  BIOL (Biological study); PRP (Properties); USES
       (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
               8 REFERENCES IN FILE CA (1907 TO DATE)
               8 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> d his

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     FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
     14:53:07 ON 08 JUN 2004
L1            35 S (AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE)
L2            13 DUP REM L1 (22 DUPLICATES REMOVED)

     FILE 'REGISTRY' ENTERED AT 14:55:52 ON 08 JUN 2004
L3             6 S AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE

=> s l2 and dna
L2 CANNOT BE SEARCHED IN REGISTRY
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this L-number.

=> file medline caplus biosis biotechds embase scisearch
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                                                   ENTRY    SESSION
FULL ESTIMATED COST                                43.31      95.07

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     (FILE 'HOME' ENTERED AT 14:52:10 ON 08 JUN 2004)

     FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
     14:53:07 ON 08 JUN 2004
L1            35 S (AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE)
L2            13 DUP REM L1 (22 DUPLICATES REMOVED)

     FILE 'REGISTRY' ENTERED AT 14:55:52 ON 08 JUN 2004
L3             6 S AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE

     FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
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=> s l2 and dna
L4            5 L2 AND DNA

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5             5 DUP REM L4 (0 DUPLICATES REMOVED)

=> d l5 1-5 ibib ab

L5   ANSWER 1 OF 5  CAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:609986  CAPLUS
DOCUMENT NUMBER:          139:160786
TITLE:                    Biosynthesis of isopentenyl pyrophosphate using
                          recombinant microbial metabolic pathways
INVENTOR(S):              Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim,
                          Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo;
                          Newman, Jack; Khlebnikov, Artem Valentinovich
PATENT ASSIGNEE(S):       USA
SOURCE:                   U.S. Pat. Appl. Publ., 40 pp.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   2
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.   DATE
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     US 2003148479      A1   20030807       US 2001-6909      20011206
     US 2004005678      A1   20040108       US 2003-411066    20030409
PRIORITY APPLN. INFO.:                   US 2001-6909      A2 20011206
AB   Methods for synthesizing isopentenyl pyrophosphate are provided.  A first
     method comprises introducing into a host microorganism a plurality of
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heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. A related method comprises introducing into a host microorganism an intermediate in the mevalonate pathway and at least one heterologous nucleic acid sequence, each sequence coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into isopentenyl pyrophosphate. The invention also provides nucleic acid sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

L5 ANSWER 2 OF 5 MEDLINE on STN
ACCESSION NUMBER: 2001197498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11289612
TITLE: **Amorpha-4,11-diene synthase**: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin.
AUTHOR: Wallaart T E; Bouwmeester H J; Hille J; Poppinga L; Maijers N C
CORPORATE SOURCE: GenoClipp Biotechnology BV, Meditech Center, Groningen, The Netherlands.. mail@genoclipp.com
SOURCE: Planta, (2001 Feb) 212 (3) 460-5.
Journal code: 1250576. ISSN: 0032-0935.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY006482
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719

AB The sesquiterpenoid artemisinin, isolated these from the plant Artemisia annua L., and its semi-synthetic derivatives are a new and very effective group of antimalarial drugs. A branch point in the biosynthesis of this compound is the cyclisation of the ubiquitous precursor farnesyl diphosphate into the first specific precursor of artemisinin, namely amorpha-4,11-diene. Here we describe the isolation of a cDNA clone encoding **amorpha-4,11-diene synthase**. The deduced amino acid sequence exhibits the highest identity (50%) with a putative sesquiterpene cyclase of A. annua. When expressed in Escherichia coli, the recombinant enzyme catalyses the formation of amorpha-4,11-diene from farnesyl diphosphate. Introduction of the gene into tobacco (Nicotiana tabacum L.) resulted in the expression of an active enzyme and the accumulation of amorpha-4,11-diene ranging from 0.2 to 1.7 ng per g fresh weight.

L5 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:144616 CAPLUS
DOCUMENT NUMBER: 132:204840
TITLE: Artemisia annua **amorpha-4, 11-diene synthase**, its cDNA, recombinant expression, and methods of amorpha-4,11-diene and artemisinin synthesis via transgenic plants
INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan
PATENT ASSIGNEE(S): Neth.
SOURCE: Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

EP 982404 A1 20000301 EP 1998-202854 19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
CA 2340925 AA 20000309 CA 1999-2340925 19990827
WO 2000012725 A2 20000309 WO 1999-EP6302 19990827
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
AU 9957423 A1 20000321 AU 1999-57423 19990827
AU 766764 B2 20031023
EP 1108041 A2 20010620 EP 1999-944535 19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
BR 9913196 A 20010925 BR 1999-13196 19990827
JP 2002523101 T2 20020730 JP 2000-567711 19990827
ZA 2001001455 A 20010828 ZA 2001-1455 20010221
PRIORITY APPLN. INFO.: EP 1998-202854 A 19980827
WO 1999-EP6302 W 19990827

AB **Amorpha-4,11-diene synthase** from Artemisia annua L., its cDNA, recombinant expression, and methods of prepg. amorpha-4,11-diene and artemisinin from farnesyl pyrophosphate (FPP) using transgenic organism are provided. Amorpha-4,11-diene is a precursor of the new anti-malarial drug artemisinin produced by the plant Artemisia annua L. A cDNA encoding **amorpha-4,11-diene synthase** from A. annua has been isolated and sequenced, and the corresponding amino acid sequence has been detd. Recombinant **amorpha-4, 11-diene synthase** expressed in E. coli, transgenic tobacco, and transgenic A. annua catalyzed conversion of FPP into amorpha-4,11-diene. Further conversion of amorpha-4,11-diene into artemisinin was obsd. in transgenic A. annua. The invention may be useful in obtaining enhanced prodn. of stereochem. desirable artemisinin.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 5 MEDLINE on STN
ACCESSION NUMBER: 2001128077 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11185551
TITLE: **Amorpha-4,11-diene synthase** of Artemisia annua: cDNA isolation and bacterial expression of a terpene synthase involved in artemisinin biosynthesis.
AUTHOR: Chang Y J; Song S H; Park S H; Kim S U
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, Korea.
SOURCE: Archives of biochemistry and biophysics, (2000 Nov 15) 383 (2) 178-84.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ251751
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial

sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express **amorpha-4,11-diene synthase** for metabolic engineering. PCR amplification of genomic **DNA** with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp **DNA** fragment. This fragment was used in Northern blot analysis as a probe, showing approximately 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A soluble fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS analysis as amorpha-4,11-diene.

L5 ANSWER 5 OF 5 MEDLINE on STN
ACCESSION NUMBER: 2000479808 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11032404
TITLE: Molecular cloning, expression, and characterization of **amorpha-4,11-diene synthase**, a key enzyme of artemisinin biosynthesis in Artemisia annua L.
AUTHOR: Mercke P; Bengtsson M; Bouwmeester H J; Posthumus M A; Brodelius P E
CORPORATE SOURCE: Department of Plant Biochemistry, Lund University, Sweden.
SOURCE: Archives of biochemistry and biophysics, (2000 Sep 15) 381 (2) 173-80.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF138959
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001031

AB In plants, sesquiterpenes of different structural types are biosynthesized from the isoprenoid intermediate farnesyl diphosphate. The initial reaction of the biosynthesis is catalyzed by sesquiterpene cyclases (synthases). In Artemisia annua L. (annual wormwood), a number of such sesquiterpene cyclases are active. We have isolated a cDNA clone encoding one of these, **amorpha-4,11-diene synthase**, a putative key enzyme of artemisinin biosynthesis. This clone contains a 1641-bp open reading frame coding for 546 amino acids (63.9 kDa), a 12-bp 5'-untranslated end, and a 427-bp 3'-untranslated sequence. The deduced amino acid sequence is 32 to 51% identical with the sequence of other known sesquiterpene cyclases from angiosperms. When expressed in Escherichia coli, the recombinant enzyme catalyzed the formation of both olefinic (97.5%) and oxygenated (2.5%) sesquiterpenes from farnesyl diphosphate. GC-MS analysis identified the olefins as (E)-beta-farnesene (0.8%), amorpha-4,11diene (91.2%), amorpha-4,7(11)-diene (3.7%), gamma-humulene (1.0%), beta-sesquiphellandrene (0.5%), and an unknown olefin (0.2%) and the oxygenated sesquiterpenes as amorpha-4-en-11-ol (0.2%) (tentatively), amorpha-4-en-7-ol (2.1%), and alpha-bisabolol (0.3%) (tentatively). Using geranyl diphosphate as substrate, **amorpha-4,11-diene synthase** did not produce any monoterpenes. The recombinant enzyme has a broad pH optimum between 7.5 and 9.0 and the Km values for farnesyl diphosphate, Mg2+, and Mn2+ are 0.9, 70, and 13 microM, respectively, at pH 7.5. A putative reaction mechanism for **amorpha-4,11-diene synthase** is suggested.

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     FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
     14:53:07 ON 08 JUN 2004
L1            35 S (AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE)
L2            13 DUP REM L1 (22 DUPLICATES REMOVED)

     FILE 'REGISTRY' ENTERED AT 14:55:52 ON 08 JUN 2004
L3             6 S AMORPHADIENE SYNTHASE OR AMORPHA-4 11-DIENE SYNTHASE

     FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, EMBASE, SCISEARCH' ENTERED AT
     14:58:00 ON 08 JUN 2004
L4             5 S L2 AND DNA
L5             5 DUP REM L4 (0 DUPLICATES REMOVED)

=> log
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      13.92     108.99

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                         ENTRY    SESSION
CA SUBSCRIBER PRICE                                      -1.39      -4.16

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